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No public transcriptome tests dehydroepiandrosterone at a physiological concentration: a systematic re-analysis of GEO

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Research Article

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No public transcriptome tests dehydroepiandrosterone at a physiological concentration: a systematic re-analysis of GEO

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Abstract

Background. Dehydroepiandrosterone (DHEA) is the most abundant circulating steroid in humans and the only major adrenal steroid whose level falls steeply and monotonically with age, from about 23 nM serum DHEA at age 20–30 to about 4.5 nM at age 70–80 in men. A receptor able to transduce that decline would need a half-maximal effective concentration inside roughly 5–50 nM. No such receptor has been established.

Methods. All Gene Expression Omnibus series returned by a DHEA query were retrieved (n = 43), their treatment metadata curated from series-matrix headers, and the stated DHEA concentration compared with the physiological serum-DHEA window. The datasets with replication were then re-analysed: GSE3627 (LNCaP, 300 nM, 48 h), GSE109021 (LAPC4, 20 µM, 28 compounds across 3 days), GSE290297 (mouse Th17, 50 µM, n = 5 per stratum), with GSE56240, GSE56018, GSE103844 and the GSE56019 RORγt ChIP-seq used as reference sets.

Results. Of the 43 series, 28 administer DHEA, 12 deposit a dose or concentration, and 5 state an in vitro concentration. None treats cells inside the physiological window. The lowest concentration in the public record is 300 nM (GSE3627), 13-fold above the young-adult serum level and 67-fold above the level at age 70–80; the median is 10 µM, 435-fold above the young-adult level. In androgen-receptor-

positive cells the DHEA response is not separable from the androgen receptor response: in GSE3627 the variance of the DHEA-minus-DHT difference is −0.00102 (95% CI −0.00209 to +0.00007) against a DHT-response variance of +0.04445, and in GSE109021 DHEA behaves as a partial androgen-receptor antagonist (α = 0.631 ± 0.105; 62.1% of its response variance explained by the androgen-receptor axis) with no residual component above a potency-matched band (0.305 against 0.431 ± 0.126, rank 4 of 13, p = 0.769). In Th17 cells, where *Ar* transcript is effectively absent (0.134 TPM), 50 µM DHEA still moves 2230–4351 genes per stratum and suppresses the IL-17 effector module; the response projects onto a mouse *Rorc*-knockout signature (r = +0.422 whole signature; +0.721 restricted to the six effector genes; +0.189 for the remaining 54, p = 0.004) where an unrelated active compound, EGCG, does not (+0.028, +0.417, −0.161). Against RORγt ChIP-seq peaks, however, the DHEA effect does not reach direct targets: in an expression-matched test the *Rorc* knockout is displaced below its matched control (observed −0.0087 against +0.0085, p = 0.014) while DHEA is displaced in the opposite direction, indistinguishably from solvent.

Conclusion. Every DHEA transcriptional effect in the public record was measured at 13- to 2174-fold above the physiological serum DHEA concentration. The concentration window in which the age-related decline could be transduced has never been assayed. The failure of these datasets to identify a DHEA receptor is therefore uninformative about its existence, and the screen specifies the experiment that would be informative.

Introduction

Dehydroepiandrosterone and its sulphate are the most abundant steroids in human circulation, and they are the only major adrenal steroids whose concentration falls steeply and monotonically across adult life. Serum DHEA is 22.7 ± 1.7 nM in men and 23.8 ± 3.4 nM in women at age 20–30, and falls to 4.5 ± 0.4 nM in men and 7.2 ± 1.5 nM in women at age 70–80, declines of 80.1% and 69.7% respectively [1]. DHEA sulphate runs in the low micromolar range and falls in parallel. These are total serum concentrations; the unbound fraction is a few percent of that, so a comparison of nominal medium concentrations with these values is the conservative one. The decline tracks involution of the adrenal zona reticularis and loss of cytochrome b5 [3,4], not a change in downstream steroid demand, and it is not shared by cortisol or aldosterone.

This pattern has sustained a long search for a receptor that would make the decline physiologically meaningful. Most of the molecular effects attributed to DHEA, how-

ever, are explained by its conversion to testosterone and dihydrotestosterone in peripheral tissue, so that DHEA acts as a prohormone rather than a ligand [2]. Proposals for a receptor acting on DHEA itself include the sigma-1 receptor, the translocator protein TSPO, peroxisome-proliferator-activated receptors, G-protein-coupled candidates, and direct modulation of neurotransmitter receptors. None is established, and the reported affinities are mostly micromolar.

A receptor that transduces the age-related decline is constrained by that decline. For a change from 23 nM to 4.5 nM to move a transcriptional output, the receptor must operate with a half-maximal effective concentration of roughly 5–50 nM: much above that window and both the young and the old concentration sit on the flat low end of the curve; much below it and both saturate. This is a sharp and testable criterion, and it applies to any experiment that claims to characterise a DHEA receptor.

Public transcriptome repositories are the natural place to apply it, because they record both the response and the concentration that produced it. We therefore asked two questions in sequence. First, at what concentrations has DHEA actually been applied in the public record, and does any dataset fall inside the physiological window? Second, in the datasets that do have replication, what receptor can the response be attributed to? The two questions turn out to be connected: the attribution that the data support is bounded by the concentration at which the data were collected.

Methods

Dataset retrieval and concentration curation

GEO was queried through E-utilities for series mentioning dehydroepiandrosterone or DHEA, returning 43 series. For each, the series-matrix header was downloaded from the NCBI FTP site and the fields `!Series_title`, `!Series_summary`, `!Series_overall_design`, `!Sample_title`, `!Sample_source_name_ch1`, `!Sample_characteristics_ch1`, `!Sample_treatment_protocol_ch1`, `!Sample_growth_protocol_ch1` and `!Sample_description` were extracted. Each series was then classified by hand into one of: in vitro DHEA addition, in vivo DHEA dosing, human DHEA administration, DHEA measured but not administered, DHEA produced but not administered, incidental mention, or false hit. Where a concentration or dose was stated anywhere in those fields it was recorded verbatim and converted to nanomolar for in vitro work. Concentrations were expressed as multiples of the mean total serum DHEA level at age 20–30 (23.0 nM, the mean of the male and female values) and of the male level at age 70–80 (4.5 nM) [1].

Expression data and normalisation

Affymetrix series were taken as deposited VALUE columns with ABS_CALL where present, quantile-normalised across arrays, and log2-transformed. RNA-seq series were taken as deposited counts or FPKM/TPM, filtered to genes detected in every sample of the stratum being tested, and log2-transformed after a pseudocount. Probe-to-gene mapping used the platform annotation deposited with each series. Contrasts were formed within each independent replicate, batch or culture day before any pooling, so that a control array is never shared between two contrasts that are afterwards compared; sharing a control inflates or inverts the correlation between two difference vectors and was the single most common artefact encountered.

Variance components

For GSE3627, the reproducible component of a response was estimated by decomposing the per-probe difference vectors across independent experiments. Writing the DHEA and DHT responses in experiment e as A[e] and T[e], the vehicle-free contrast g[e] = A[e] − T[e] isolates the part of the DHEA response that is not the androgen-receptor response, and its between-experiment covariance estimates the reproducible variance of that part while the within-experiment scatter estimates array noise. A model including a vehicle term was rejected because it returned an out-of-range correlation. Confidence intervals came from a probe bootstrap (10,000 resamples) and the null from label permutation.

Androgen-receptor axis and residual fitting

For GSE109021, the androgen-receptor axis was defined not as a projection direction but as a reproducible probe set: probes moving consistently in the same direction under the strong agonists across all culture days (583 probes). The response of each compound was read out as the mean fold change over that set, giving a scalar α relative to the full agonist. A whole-transcriptome projection was also computed and rejected, because the reference arrays shared between compounds forced every compound, including the full agonists, to a negative loading. The residual after removing the androgen-receptor component was compared with a band of potency-matched compounds (compounds whose α lies within a fixed window of the test compound) to ask whether any non-androgen-receptor component is present.

Differential expression and multiple testing

Differential expression used Welch's t-test within stratum. Benjamini–Hochberg q-values [14] were computed over finite p-values only; genes with zero variance give an undefined p-value, and propagating those through the step-up procedure silently returns an all-undefined q-vector, which was the cause of one erroneous intermediate result during this work. The proportion of true nulls was estimated from the p-value histogram as a check on each stratum.

Signature projection

RORγt-loss reference signatures were built from GSE56240 and GSE56018 (mouse *Rorc* knockout) [9] and GSE103844 (human *RORC* siRNA with a *RORA* siRNA arm as an off-target control) [10]. A genome-wide signature did not transfer across species or platform: direction agreement over all genes was 50.1%, 50.7% and 49.1%, that is, at chance. Projections were therefore restricted to the top-effect genes of the reference, with the number of genes varied (200 and 500) as a sensitivity analysis, and significance taken from 20,000 label permutations. The Th17 effector genes were then removed and the projection recomputed, to ask whether anything beyond that module is shared.

ChIP-seq peak assignment

RORγt ChIP-seq peaks from GSE56019 [9] were used. The genome build was not stated in the deposited files and was inferred from the maximum peak coordinate per chromosome, which is compatible with mm9 and incompatible with mm10. Peaks were assigned to the nearest transcription start site, and the assignment validated by checking that known RORγt targets recover their peaks. A gene was called bound if a peak lay within the stated distance of its transcription start site. To ask whether a response reaches direct targets, bound genes were compared with a control set sampled to match their expression distribution, since expression level alone predicts both binding and response magnitude. Significance came from 10,000 matched resamples, one-sided for displacement below the matched control. Sensitivity of the test was calibrated by subsampling the *Rorc* knockout effect until the test lost significance, giving the fraction of full-knockout efficiency the design can detect.

Software and environment

Analysis used Python 3 with NumPy, SciPy, pandas and Matplotlib. All downloaded archives were extracted into isolated directories and read by scripts held elsewhere,

with the interpreter run in isolated mode, so that no code could be loaded from a downloaded directory.

Results

No public dataset applies DHEA at a physiological concentration

Of the 43 DHEA-related GEO series, 42 could be classified from the deposited headers; GSE68387 is a SuperSeries whose dose is not in the header. Only 28 of the 43 administer DHEA at all. The remainder measure or produce DHEA without giving it (13 series), and one is a false hit: GSE160086 concerns docosahexaenoyl ethanolamide, not the steroid.

Of the 28 series that administer DHEA, 12 deposit a dose or concentration and 16 deposit none. Five state an in vitro concentration, and these five are the entire public record of DHEA applied to cells at a known concentration (Table 1).

Table 1: Every GEO series stating an in vitro DHEA concentration.

Series	System	Stated conc.	× young adult (23.0 nM)	× age 70–80 (4.5 nM)	n
GSE3627	LNCaP, 48 h	300 nM	13	67	9
GSE216991	iPSC neurons, compound library	10 µM	435	2222	1441
GSE261582	*Arabidopsis* leaves	10 µM	435	2222	12
GSE109021	LAPC4, 6 h	20 µM	870	4444	96
GSE290297	mouse Th17, 72 h	50 µM	2174	11111	79

No series treats cells inside the physiological serum-DHEA window of 4.5–23 nM, and none comes within a factor of ten of it. The lowest concentration anywhere in the public record is 300 nM, 13-fold above the young-adult serum level and 67-fold above

the level at age 70–80. The median stated concentration is 10 µM, 435-fold above the young-adult level.

The in vivo series are less directly comparable because tissue exposure is not recoverable from the dose, but they occupy the same range: where a dose is stated it is 40–60 mg/kg/day (GSE316046, GSE195605, GSE343214), 600 mg/kg (GSE68110), or 750 ppm in the diet (GSE7837). Seven further series use DHEA to build a polycystic-ovary-syndrome model, a protocol selected for supraphysiological androgen exposure rather than for receptor pharmacology.

One series states its intent explicitly: GSE218045 uses DHEA as an inhibitor of glucose-6-phosphate dehydrogenase and of glucose metabolism, which is a known non-receptor action of the compound in the micromolar range [5]. That action is available at every concentration used in the five in vitro series above, and at none of the concentrations present in human serum.

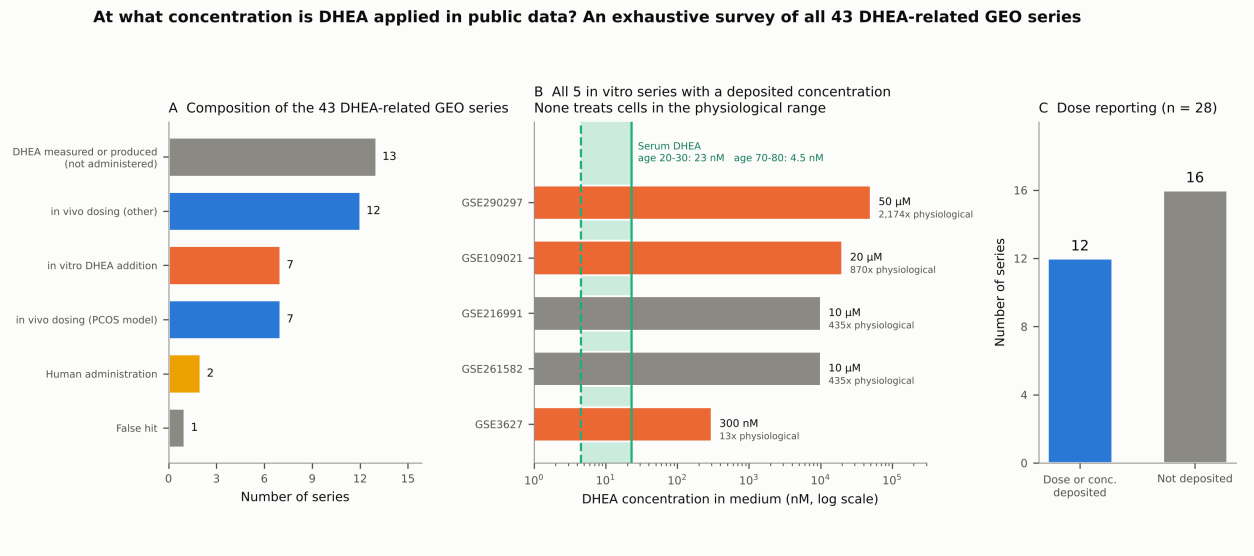


Figure 1: Concentration screen of all 43 DHEA-related GEO series. (A) Category breakdown. (B) The five series stating an in vitro concentration, on a log scale, with the physiological serum-DHEA band (4.5–23 nM) shaded; orange marks the series re-analysed here. (C) Of the 28 series that administer DHEA, how many deposit a dose at all.

In androgen-receptor-positive cells the DHEA response is the androgen-receptor response

GSE3627: the DHEA response and the DHT response are not separable

GSE3627 treated LNCaP cells with 300 nM DHEA or 300 nM dihydrotestosterone for 48 h [6]. If DHEA acted through any receptor other than the androgen receptor, the

difference between the two responses would contain a reproducible component. It does not. The variance of the reproducible part of the DHEA-minus-DHT difference is −0.00102, with a bootstrap 95% confidence interval of −0.00209 to +0.00007, that is, indistinguishable from zero and bounded above by a value two orders of magnitude below the signal it would have to exceed. The same decomposition applied to the DHT response itself returns +0.04445, with a permutation z of +54.5. The estimate of the difference variance remained at or below zero in all nine sensitivity settings tried (probe filtering, normalisation, bootstrap unit, and the number of experiments pooled).

Read out over androgen-receptor target probes, the ratio of the DHEA effect to the DHT effect is 1.090 (bootstrap 95% CI 1.014–1.185). At 300 nM in a cell line that expresses the full conversion machinery, DHEA reproduces the dihydrotestosterone response in magnitude as well as in direction.

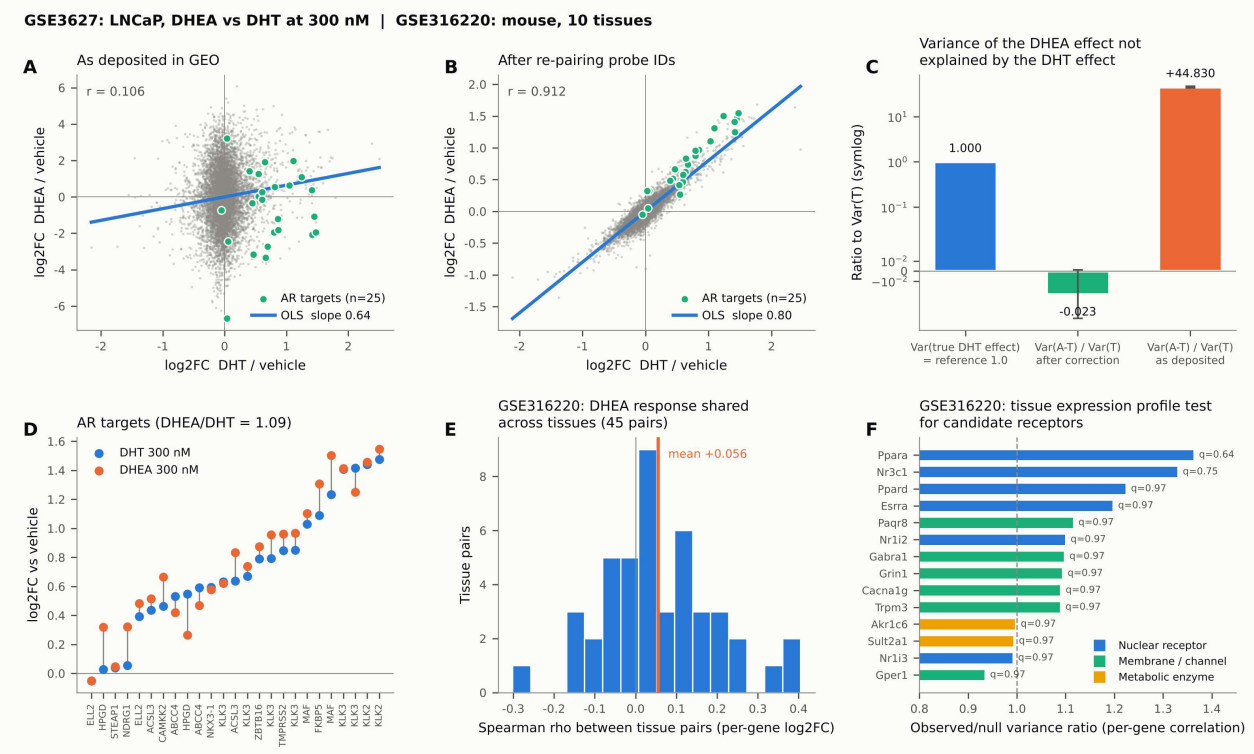


Figure 2: GSE3627 (LNCaP, 300 nM, 48 h). Variance components of the DHEA-minus-DHT difference against the DHT response, with bootstrap confidence intervals and the permutation null, and the androgen-receptor-target readout.

GSE109021: DHEA is a partial androgen-receptor antagonist with no residual component

GSE109021 treated LAPC4 cells for 6 h with 28 compounds at 20 µM in the presence of 0.33 nM R1881, across three culture days [7]. In that design DHEA acts as a partial antagonist of the androgen receptor, with $\alpha = 0.631 \pm 0.105$ relative to the full agonist (relative activity 0.313). This is consistent with the GSE3627 result rather than in conflict with it: a weak partial agonist behaves as an agonist when given alone and as an antagonist when given against a strong agonist.

The question the design can answer is whether anything is left over. The androgen-receptor axis explains 62.1% of the variance of the DHEA response. The residual, at 0.305, is not larger than the residual of potency-matched compounds, which average 0.431 ± 0.126; DHEA ranks 4th of the 13 compounds in that band, $p = 0.769$. In a multi-axis fit DHEA loads only on the androgen-receptor axis ($+0.744$, $p = 0.003$) and on no other. Its ratio of androgen-receptor-specific to non-specific effect is 2.449, which ranks 5th of 28 compounds, so the response is receptor-directed rather than a general toxicity.

Taken together, the two androgen-receptor-positive datasets place an upper bound on any non-androgen-receptor DHEA action in those cells: at 300 nM and at 20 µM, after the androgen-receptor component is removed, nothing reproducible remains.

In androgen-receptor-negative Th17 cells, DHEA suppresses the IL-17 effector module

GSE290297 treated differentiating mouse Th17 cells with 50 µM DHEA against a methanol vehicle, and with EGCG against DMSO, in five independent cultures [8]. EGCG serves as a control for the generic transcriptional consequences of adding an active small molecule at high concentration.

In these cells *Ar* is effectively absent at 0.134 TPM, and the enzymes that would convert DHEA to an androgen are likewise absent. Any response here is therefore not the androgen-receptor response characterised above. A response is present: 2230 to 4351 genes reach a false-discovery rate below 0.05 depending on the stratum, and 55.5% of the total variance of the DHEA contrast is reproducible across the five cultures (95% CI 51.7–59.8%), against 50.7% for EGCG and 35.7% for the vehicle-versus-vehicle contrast.

The direction of the response is specific. DHEA suppresses *Il17a*, *Il17f*, *Il22*, *Il23r* and *Il1r1*, and raises *Gata3* by +1.55 to +1.94 log2 units in all four strata. *Rorc* transcript

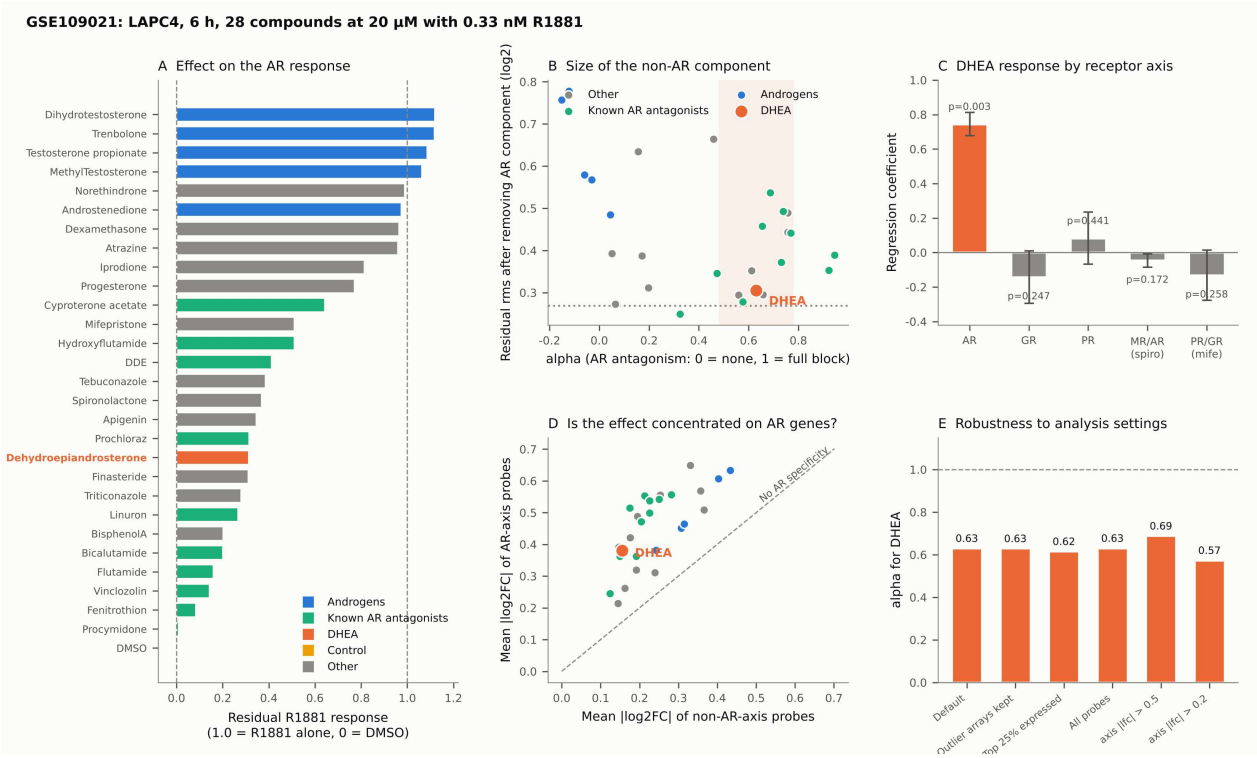


Figure 3: GSE109021 (LAPC4, 20 µM, 28 compounds, 3 culture days). Androgen-receptor activity α per compound, the residual after removing the androgen-receptor component against the potency-matched band, and the multi-axis loadings for DHEA.

itself is unchanged. EGCG moves a different program, dominated by ATF4, heat-shock and glycolytic genes.

Projected onto a mouse *Rorc*-knockout signature, DHEA and EGCG separate cleanly (Table 2).

Table 2: Projection of each response onto the 60-gene mouse *Rorc*-knockout signature.

Query	Whole 60-gene signature	Six Th17 effector genes	Remaining 54 genes
DHEA	r = +0.422 (p < 0.0001)	r = +0.721 (p < 0.0001)	r = +0.189 (p = 0.004)
EGCG	r = +0.028 (p = 0.48)	r = −0.067 (p = 0.0003)	r = −0.161 (p = 0.92)
Vehicle	r = −0.146 (p = 1.0)	r = +0.681 (p = 0.94)	r = −0.289 (p = 1.0)

The comparison with the reference sets also constrains what a RORγt-loss signature can mean. A genome-wide signature does not transfer: direction agreement between the mouse knockout and the human siRNA references is 50.1%, 50.7% and 49.1% across the three pairings, that is, at chance. The reliably shared core reduces to *Il17a* and *Il17f*. Restricted to top-effect genes the projection is informative, and a *RORA* siRNA arm included as an off-target control is negative for DHEA (r = −0.004, 38% direction agreement), which excludes a generic siRNA or stress signature as the explanation.

So in a cell without the androgen receptor, 50 µM DHEA produces a transcriptional state resembling loss of RORγt, concentrated in the IL-17 effector module and not reproduced by an unrelated active compound.

The DHEA effect does not reach direct RORγt targets

A transcriptional state that resembles loss of RORγt can arise either at RORγt itself or downstream of it. RORγt ChIP-seq distinguishes the two, because an effect at the factor should be concentrated at the genes it binds.

Peaks were taken from GSE56019 (2257 peaks in Th17 cells) [9]. The genome build is not stated in the deposited files; the maximum peak coordinate per chromosome is compatible with mm9 and incompatible with mm10, and the assignment was vali-

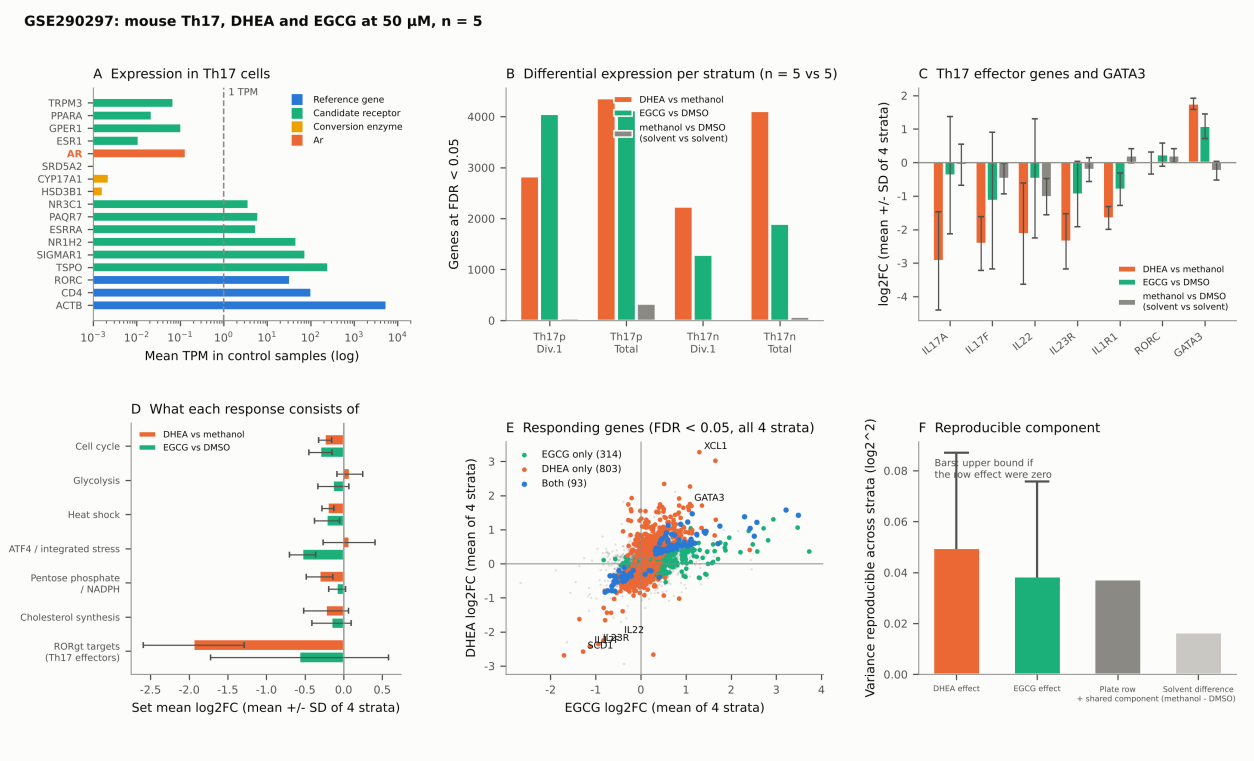


Figure 4: GSE290297 (mouse Th17, 50 µM, n = 5). *Ar* and conversion-enzyme expression, the reproducible variance fraction of each contrast, the differentially expressed gene counts per stratum, and the Th17 effector and *Gata3* changes for DHEA against EGCG.

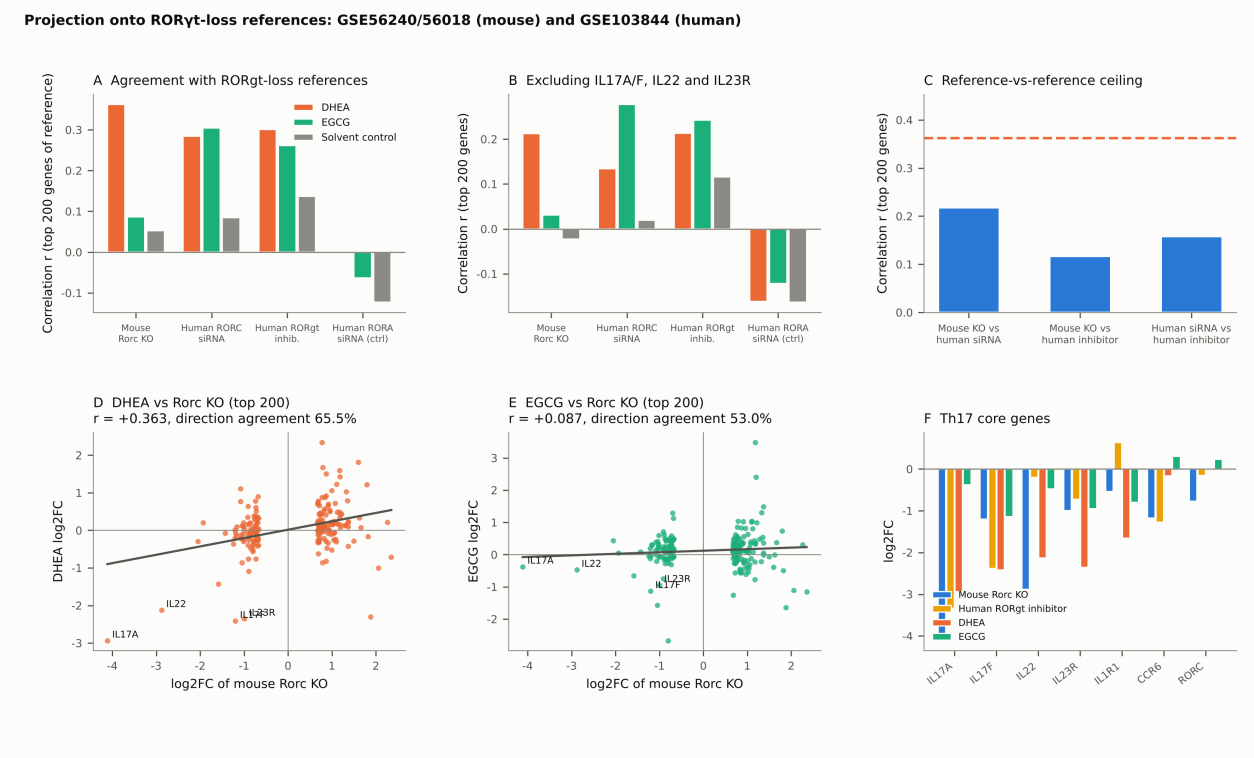


Figure 5: Projection onto RORγt-loss reference signatures. Genome-wide direction agreement across species and platform, and the top-effect projection for DHEA, EGCG and vehicle against the mouse *Rorc* knockout, the human *RORC* siRNA and the *RORA* off-target control, with and without the Th17 effector genes.

dated by recovering peaks at known targets, including a peak 0.1 kb from the *Il17a* transcription start site.

Because expression level predicts both binding and response magnitude, bound genes were compared with controls sampled to match their expression distribution. Under that test the *Rorc* knockout behaves as a positive control should and DHEA does not (Table 3).

Table 3: Expression-matched displacement of RORγt-bound genes.

Response	Peak set	n bound	Observed	Matched control	p
Rorc knockout	RORγt Th17	1211	−0.0087	+0.0085	0.014
DHEA	RORγt Th17	1105	+0.0751	+0.0109	1.0
EGCG	RORγt Th17	1105	+0.1247	+0.1077	0.98
Vehicle	RORγt Th17	1105	+0.0444	+0.0184	1.0

The knockout displaces bound genes downward relative to matched controls. DHEA displaces them upward, in the same direction and to a similar degree as the vehicle. Foxp3 peaks, included as an unrelated factor, are not displaced for any response.

A conditional test gives the same answer with more resolution. Among the 175 genes that fall in the *Rorc* knockout, the 33 that carry a RORγt peak show a mean DHEA log2 fold change of -0.349 against -0.050 for the unbound ones. Removing the five-gene IL-17 module (*Il17a*, *Il17f*, *Il23r*, *Il1r1* and one adjacent gene) abolishes the difference entirely: -0.058 against -0.050, $p = 0.726$. The apparent enrichment at bound genes is the IL-17 module and nothing else.

This is not a power failure. Subsampling the knockout effect shows that the test retains significance down to 62% of full-knockout efficiency, so an effect at RORγt of more than roughly two-thirds the magnitude of deleting the gene would have been detected.

DHEA therefore produces a *Rorc*-knockout-like downstream state without acting at RORγt [11]. Its effect enters at the IL-17 effector module, which leaves the upstream inputs to that module, such as *Il23r* to STAT3 and *Il1r1* to IRF4, as the places to look.

A probe-pairing defect in GSE3627 inverts the deposited conclusion

The GSE3627 result above required a correction to the deposited data, which is reported here because the defect is invisible to ordinary quality control and because its

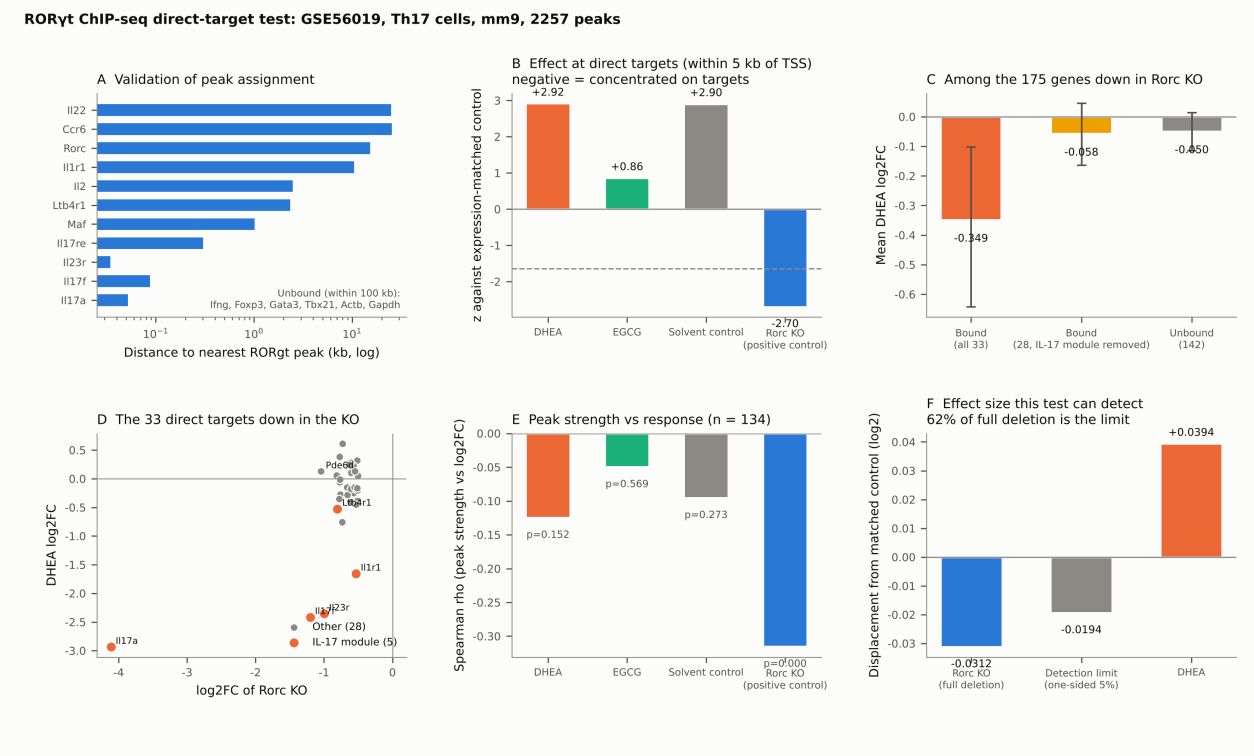


Figure 6: RORγt ChIP-seq direct-target test (GSE56019, mm9). Peak-to-transcription-start-site assignment and its validation, the expression-matched displacement test for each response, the conditional test among *Rorc*-knockout-down genes with and without the IL-17 module, and the sensitivity calibration against subsampled knockout efficiency.

effect is to reverse the biological conclusion.

Two of the nine arrays, GSM83827 and GSM83837, carry probe identifiers sorted in ASCII order while their VALUE column retains the original chip order. The two columns are therefore misaligned row by row. No summary statistic flags this: the arrays have normal intensity distributions, normal present-call rates by count, and pass the usual outlier checks. What exposes it is correlation with the other arrays of the same experiment, which is r = 0.02 for these two against r > 0.97 for the rest, and the ABS_CALL concordance, which is 0.52 for these two, that is, chance.

Re-pairing each value to the chip order restores both: correlation becomes 0.988 and 0.975, and ABS_CALL concordance becomes 0.897 and 0.871. The check is biology-free, since ABS_CALL concordance depends only on probe identity.

The consequences are not small. Analysed as deposited, the variance of the reproducible DHEA-minus-DHT difference is 44.8 times the variance of the DHT response itself, and the ratio of the DHEA to the DHT effect over androgen-receptor targets is −0.93. Both numbers support the conclusion that DHEA acts through something other than the androgen receptor. After re-pairing, the same statistics are −0.00102 and +1.090 respectively, and support the opposite conclusion. The sign of the finding is set by a bookkeeping error in two of nine files.

Two practical points follow. First, any re-analysis that pools arrays across a deposited series should check probe-identity concordance, not only intensity distributions, before pooling. Second, a difference-of-differences design is unusually exposed to this class of defect, because misalignment destroys the correlation structure that the design relies on while leaving the marginal distributions intact.

Discussion

The three re-analyses give a consistent account of what DHEA does in the public record, and the concentration screen says what that account is worth.

Where the androgen receptor is present, DHEA is an androgen-receptor ligand and nothing else that the data can detect. Where it is absent, DHEA at 50 µM suppresses the IL-17 effector module and produces a state resembling loss of RORγt, but it does so without acting at RORγt, and 50 µM is more than three orders of magnitude above the concentration in serum. The non-receptor actions of DHEA that are already established, including inhibition of glucose-6-phosphate dehydrogenase [5], operate in exactly that range; one deposited series uses the compound for that purpose. The Th17 effect is therefore more parsimoniously read as a micromolar action than as

evidence of a high-affinity receptor.

The broader point is methodological. The argument for a DHEA receptor rests on the age-related decline, and that decline sets the concentration window any candidate receptor must operate in. The public transcriptome record contains no measurement inside that window, and none within a factor of ten of it. The lowest concentration ever deposited, 300 nM, already saturates a receptor with a half-maximal effective concentration anywhere in the physiological range; at that concentration a receptor whose occupancy differs between a 25-year-old and a 75-year-old is fully occupied in both. It follows that the failure of these datasets to identify a receptor is uninformative about whether one exists. They were not measurements of the question.

This also explains a pattern in the literature that is otherwise puzzling: reported DHEA effects are numerous, reproducible within their own systems, and mutually unrelated. Micromolar concentrations of a lipophilic steroid produce effects, and different cell types produce different ones. A screen that distinguishes receptor-mediated from concentration-driven effects has not been run because the concentrations that would permit the distinction have not been used.

The screen specifies the experiment. A dose series spanning roughly 1 to 100 nM, in charcoal-stripped serum so that the background DHEA concentration is known, in cells lacking the conversion enzymes and the androgen receptor, is the minimum design that can test the hypothesis the age decline motivates. A graded transcriptional response across 4.5 to 23 nM would be the first direct evidence for a receptor that transduces the decline; a flat response across that range, with effects appearing only above 1 µM, would show that the decline is not transduced at the transcriptional level and would redirect the question to non-genomic or prohormone mechanisms. Either result is informative, which is not true of any dataset now public.

Several limitations apply. The screen covers GEO, not every repository, and relies on concentrations as deposited; 16 of 28 series that administer DHEA state no dose at all, so the record is incomplete as well as skewed. The serum DHEA reference values are population means from a single cohort of 60 subjects, and tissue concentrations are not serum concentrations; nominal medium concentrations are also not free concentrations, since medium protein binds the steroid. The Th17 finding rests on a single series, and the RORγt reference signatures do not transfer genome-wide, which limited the projection to top-effect genes. Finally, a transcriptional readout cannot exclude a receptor whose output is non-genomic; it can only say that no transcriptional consequence has been measured where one would have to appear.

Data and code availability

All data analysed are public. The full classification of the 43 series, with the curated concentration, dose, category and note for each, is provided as Supplementary Table 1. Analysis scripts will be deposited in a public repository on acceptance.

Accession	Use in this study
GSE3627	LNCaP, DHEA and DHT at 300 nM; variance-component analysis
GSE109021	LAPC4, 28 compounds at 20 µM; androgen-receptor axis and residual fitting
GSE290297	Mouse Th17, DHEA and EGCG at 50 µM; differential expression and projection
GSE56240, GSE56018	Mouse *Rorc* knockout; reference signature
GSE103844	Human *RORC* and *RORA* siRNA; reference signature and off-target control
GSE56019	RORγt ChIP-seq (mm9); direct-target test
GSE316220	Mouse multi-tissue DHEA, n = 1; screened, not analysable
36 further series	Concentration screen only (Supplementary Table 1)

Declarations

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Competing interests. The author declares no competing interests.

Ethics. This study analysed only publicly deposited, de-identified data and required no ethical approval.

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